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The antibacterial, antiviral activities and phytochemical screening of some Sudanese medicinal plants

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Abstract

Methanolic extracts were obtained from 30 parts of 23 different plants belonging to 19 families. Most of the plants are used traditionally to treat different illnesses in Sudan. The extracts were screened for their biological activities against bacterial pathogens including *Escherichia coli*, *Staphyllococcus aureus*, and *Klebsiella pneumoniae* and two animal viruses representing two viral families, Newcastle Disease and Fowlpox Viruses. In addition, the extracts were evaluated for the presence of the major secondary metabolites classes. The highest activity recorded was obtained for *Zizyphus spina-christi* against *S. aureus* and *K. pneumoniae*. Seven of the tested extracts, showed virucidal activity of NDV while eight plant extracts exhibited high activity against replication of PV. Our results support, at least in part, the uses of most plants in ethnopharmacological claims.

Keywords: Antibacterial, antiviral, fowlpox, Newcastle disease virus, plant, Sudan.

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INTRODUCTION

SuELdan is the largest country in Africa with a diverse flora. Most of the Sudanese people in rural areas rely on traditional medicine for the treatment of many infectious diseases. Sudanese traditional medicine is characterized by a unique combination of knowledge and practices of Arabic, Islamic and African culture (El Hamidi 1970, El Kamali and El Khalifa 1997).

Infectious diseases are the world's leading cause of premature deaths (Emori and Gaynes 1993). Therefore, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action. On the other hand, viral infections are very common and responsible for a variety of infectious diseases ranging from the common cold to uniformly fatal rabies and AIDS. In contrast to the enormous amount of antimicrobial drugs, very few effective antiviral drugs are available (Vlietinck and Vanden Berghe 1991). One of the most important reasons for the lack of success in developing antiviral drugs is due to the nature of the infectious viral agents, which totally depend upon the cell they infect for their multiplication and survival (Vanden Berghe et al. 1986, Vlietinck et al. 1997). Since many of the existing disinfectants and antiseptics fail to kill all pathogenic viruses, the demand for new antiviral agents is great and needs all possible approaches towards the development of new antiviral drugs (Munro et al. 1987).

One of the possible methodologies that can be used for the discovery of antibacterial and

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antiviral principals is the screening of selected plant extracts for the activity followed by bioassay-guided fractionation of active extracts leading to the isolation of the pure constituents.

This study which is a part of a screening project, has been dedicated to investigate the antibacterial and antiviral activity of extracts from Sudanese medicinal plants. Thirty plant extracts were examined for activity against three bacterial pathogens: *Escherichia coli, Staphyllococcus aureus,* and *Klebsiella pneumoniae* and two animal viruses: Newcastle Disease Virus (NDV) and the Fowlpox Virus (PV), representing two viral families, Paramyxoviridae (RNA Virus) and Poxviridae (DNA Virus).

MATERIAL AND METHODS

Plant material

The 23 plant specimens are listed in Table 1. The 23 plant specimens were collected from different localities in Sudan; Erkowit Red Sea hills (Eastern Sudan), Kordofan (Western Sudan) and around the University of Khartoum, Khartoum state (Central Sudan) during the year 2003. Most of the plants are indigenous while some are grown and cultivated in the Sudan. The specimens were authenticated at the Department of Botany, University of Khartoum, Sudan and voucher specimens were deposited in the Botany Department Herbarium.

Test microorganisms

Bacteria: Standard bacterial strains were used for screening these were *Escherichia coli* (NCTC 8196), *Klebsiella pneumoniae* (ATCC 35657), and *Staphyllococcus aureus* (NCTC 6447).

Viruses: Two common animal viruses were used in this study, NDV (The thermostable I_2 strain, its EID_{50} is $10^{9.13}$/mL) and PV (which was prepared from infected tissues).

Preparation of crude plant extracts: The plant materials (Table 1) were air dried and grounded into a coarse powder. About a 100 g sample from each plant species was extracted with methanol (MeOH) after defattening the material with petroleum ether and chloroform. The extracts were then evaporated to dryness under reduced pressure and redissolved in MeOH to attain the required concentrations of 100 and 200 μg/mL for antibacterial uses. In the case of the antiviral assay the dried extracts were redissolved in Hank's balanced solution to prepare the test concentrations (100 and 200 μg/mL).

Antibacterial assay: The cup-plate agar diffusion method (Kavanagh 1972) was adopted. The mean diameter of growth inhibition zones in mm of the three replicates for each treatment and consequently standard deviations SD, were recorded.

Antiviral assay: Antiviral testing of the plant extract was carried out in vitro using allantoic sac or chorio-allantoic membranes (CAM) of developing chick embryos, according to the method of Rajbhandari et al. (2001) with some minor modifications. A suspension of 0.1 mL of NDV was treated with 0.1 mL of 100 μg/mL and 200 μg/mL of the extract and the 0.1 mL of the CAM suspension of PV was also treated with 0.1 mL of 100 μg/mL and 200 μg/mL of the extract. The treated viruses were incubated at 4°C for about 1 h. The treated viruses and the controls were then inoculated via CAM and the allantoic sac of 9-11 day old chick embryos for PV and NDV respectively. Hank's balanced salt solution and the virus without treatment were used as controls. Triplicate tests were carried out for each extract against each of the two viruses the results were compared to the sample without treatment.

The results of the antiviral screening of PV were recorded as follows: - (a) + + + (highly active) (b) + + indicates moderately active, (c) + (mildly active), and (d) i (inactive). These results were compared to the sample without treatment as a + ve and Hank's balanced solution as a - ve control. In case of NDV, inhibition of the hemagglutination by

Table 1. The traditional uses of plants selected for antibacterial and antiviral screening.

Botanic name	Family	Local name	Traditional uses	References
Abutilon figarianum Webb.	Malvaceae	Gargadan	Hepatoprotective, antiplasmodial and antimicrobial	Elamin 2008
Acacia nilotica (L.) Del	Fabaceae	Garad (Sunt)	Malaria, HCV, molluscicidal activity	Hussein 1982, El Tahir et al. 1999, Hussein et al. 2000
Aloe sinkitana Rey.	Liliaceae	Sabar, Kalando (Hadandawa)	Leaves: laxative, and purgative, skin diseases, constipation, anthelmintic and haemorrhoids	UPI
Aristolochia bracteolate Retz.	Aristolochiaceae	Um Galagil	Malaria, HIV-1, antitumor, scorpion bite	El Kamali and El Khalifa 1997,1999, Hussein et al. 1999, El Tahir et al. 1999, El Khalifa et al. 2006
Avicennia marina (Forssk.) Vierh.	Avicenniaceae	Mangrove	-	-
Cassia obtusifolia L.	Fabaceae	Kawal	Diuretic, HIV-1, jaundice	Dirar 1984, Hussein et al. 1999, El Khalifa et al. 2006
Caralluma retrospiciens (Ehrenb.) N. E. Br.	Apocynaceae	Karib (Hadandawa)	Animal wounds	El Gazali 1986
Cissus quadrangularis L.	Vitaceae	Alsalaa 'Iaa'	Malaria, fracture tuberculosis, anti-dote against scorpion bite, haemorrhoids	El Gazali et al. 1994, 1997, El Kamali 2009
Combretum glutinosum Perr. Ex DC.	Combretaceae	Habiel	Febrile, jaundice, malaria, bacterial infection, relieve fever, rheumatism headache, antispasmodic	El Magboul et al.1988, El Gazali et al. 1994, El Kamali 2009
Coriandrum sativum L.	Apiaceae	Kasbara	Against flatulence, reduced plasma lipids	Suliman et al. 2008
Croton zambesicus Mull. Arg.	Euphorbiaceae	Um Gelegla	Relieve cough, malaria, HIV-1, menstrual pain	El Kamali and Khalid 1996, El Kamali and El Khalifa 1997, El Kamali 2009
Diospyros mespiliformis Hochst. Ex A. DC	Ebeanaceae	Joghan	Back pain	El Ghazali 1986
Harrisonia abyssinica Oliv.	Simaroubaceae	-	Malaria	El Tahir et al. 1999
Ipomoea carnea Jacq.	Convolvulaceae	Aweer	-	-
Lavandula coronopifolia Poir.	Lamiaceae	Sedam (Hadandawa)	Astringent, antibacterial, antispasmodic	UPI
Maerua oblongifolia	Capparidaceae	Abu Tamra	Hypocholesterolmic, malaria astringent, abdominal pains	El Kamali and El Khalifa 1999 Kamali 2009

- = Not recorded
UPI = Unpublished Information

each extract was calculated as follows:

$$\text{HA inhibition } \% = \frac{C-T}{C},$$

Where, C = the base two logarithmic HA titre of the virus control, and T = the base two logarithmic HA titre treated with the extracts

Phytochemical screening

All the crude extracts were analyzed for the presence of alkaloids, saponins, triterpenes, and/or steroids, flavonoids, and tannins according to standard methods (Harborne 1973).

RESULTS AND DISCUSSION

In spite of the vast diversity of Sudanese medicinal plants, there are only a few reports on some of their antiviral activity (Hussein et al. 1999, 2000), in contrast, there are many reports regarding antibacterial, antifungal, and antimalarial activities (Al Magboul et al. 1985, 1988, Khalid et al. 1986, El Tahir et al. 1999, Elegami et al. 2001, Ali et al 2002). In this study thirty crude methanolic extracts from plants, most of them, commonly used in folk medicine in the Sudan to treat different infectious diseases, were screened for antibacterial and antiviral activity against three bacterial strains and two animal viruses belonging to two important viral families.

Preliminary antibacterial screening was carried out against three standard bacterial pathogens *E. coli, K. pneumoniae,* and *S. aureus*. Among the extracts tested about 50% showed antibacterial activity against at least one bacterial strain (Table 2). While the remaining extracts were not active against any of the test organisms. This means that

the plant part used and the type of extraction might have resulted in nil activity in the test performed for this study. Some of these plants were also screened previously against other test strains (Al Magboul et al. 1985, 1988) and were found to exhibit similar results to those obtained in this study with varying degrees of potency. The differences in potency may be due to locality of the plant species, time of collection of the plant sample, storage conditions, different sensitivity of the test strains, and method of extraction (Nimri et al. 1999). In classifying the antibacterial activity as Gram positive and Gram negative, it would generally be expected that greater number of extracts would be active against Gram positive than Gram negative (McCutcheon et al. 1992). However, in this study, all those described as active extracts were able to exert similar effects against both types of bacteria. *K. pneumoniae* was the least sensitive compared to other test bacteria, which may be due to the ability of the latter to form highly resistant capsules (Ali et al. 2001).

The extracts were considered active if they reduced or decreased the HA titre of the virus in the case of NDV and reduction of lesions formation in the case of PV. One of the inherent drawbacks of in vitro antiviral testing is the environmental sensitivity of the animal cells in a culture or in an egg embryo. Preparations which exert antiviral effects in vivo may not be detected with in vitro assays because of the extremely low concentrations of extract tolerated by cells in the artificial system. Even with this limitation, 50% of the extracts exhibited some antiviral activity

Table 2. The antibacterial activity of MeOH extracts of selected plants from Sudan.

Botanic name	Part screened	Bacterial strains I. Z.D. (M ± SD) mm					
		E.c.		*S.a.*		*K.p.*	
		100 µg/mL	200 µg/mL	100 µg/mL	200 µg/mL	100 µg/mL	200 µg/mL
Abutilon figarianum	L	10 ± 0.0	10 ± 0.0	19 ± 3.5	20 ± 1.0	20 ± 2.5	21 ± 1.0
Acacia nilotica	F	10 ± 0.3	10 ± 0.5	10 ± 0.0	10 ± 0.5	10 ± 0.0	10 ± 0.0
Aloe sinkitana	L	10 ± 0.0	10 ± 0.0	10 ± 1.2	10 ± 0.3	10 ± 0.0	11 ± 0.3
Aristolochia bracteolate	L	10 ± 0.6	10 ± 0.0	10 ± 0.3	10 ± 0.2	10 ± 0.2	10 ± 0.3
Aristolochia bracteolate	F	10 ± 0.3	12 ± 1.2	13 ± 0.0	12 ± 0.6	10 ± 0.5	10 ± 0.2
Avicennia marina	L	11 ± 0.6	14 ± 2.0	10 ± 0.2	11 ± 0.5	11 ± 0.0	13 ± 0.5
Avicennia marina	S	11 ± 1.0	13 ± 3.0	12 ± 1.2	14 ± 0.6	13 ± 1.2	14 ± 0.6
Cassia obtusifolia	L	11 ± 0.5	12 ± 0.1	12 ± 0.5	12 ± 1.2	11 ± 1.5	13 ± 0.3
Caralluma retrospiciens	WA	18 ± 1.5	19 ± 3.0	11 ± 0.6	13 ± 0.6	13 ± 0.6	14 ± 2.0
Cissus quadrangularis	WA	10 ± 0.0	10 ± 1.3	21 ± 2.0	22 ± 4.5	20 ± 0.3	20 ± 0.5
Combretum glutinosum	B	10 ± 0.2	11 ± 2.1	10 ± 0.0	10 ± 0.5	10 ± 0.6	10 ± 0.0
Coriandrum sativum	F	10 ± 0.0	10 ± 0.0	11 ± 0.3	12 ± 1.2	10 ± 0.0	13 ± 1.5
Croton zambesicus	F	10 ± 0.5	10 ± 0.0	10 ± 0.3	10 ± 0.0	10 ± 0.2	10 ± 0.0
Diospyros mespiliformis	B	16 ± 1.5	17 ± 0.6	10 ± 0.0	10 ± 0.4	10 ± 0.0	10 ± 0.0
Diospyros mespiliformis	L	18 ± 2.0	10 ± 0.5	10 ± 1.0	12 ± 0.3	10 ± 0.3	15 ± 1.5
Harrisonia abyssinica	L	10 ± 0.3	10 ± 0.5	18 ± 2.5	23 ± 2.0	14 ± 0.0	17 ± 3.5
Harrisonia abyssinica	F	18 ± 3.0	18 ± 1.2	10 ± 0.0	10 ± 0.0	10 ± 0.5	10 ± 0.3
Ipomoea carnea	L	13 ± 0.6	13 ± 0.5	17 ± 1.0	17 ± 2.5	15 ± 0.6	15 ± 1.3
Lavandula coronopifolia	WA	13 ± 1.2	16 ± 2.5	10 ± 0.0	12 ± 0.5	10 ± 0.0	10 ± 0.6
Maerua oblongifolia	L	11 ± 1.5	10 ± 0.3	12 ± 1.2	15 ± 1.0	10 ± 0.2	10 ± 0.0
Maytenus senegalensis	L	11 ± 0.5	11 ± 0.3	10 ± 0.5	10 ± 0.3	10 ± 1.0	10 ± 0.2
Maytenus senegalensis	B	18 ± 3.5	20 ± 2.0	12 ± 0.5	13 ± 1.0	12 ± 0.6	11 ± 0.0
Nigella sativa	Se	11 ± 1.2	11 ± 0.3	12 ± 0.6	14 ± 0.3	10 ± 0.3	10 ± 0.0
Prosopis chilensis	L	11 ± 0.5	12 ± 1.0	10 ± 0.0	10 ± 0.0	10 ± 0.0	12 ± 0.6
Tamarindus indica	F	18 ± 3.0	20 ± 4.0	10 ± 0.3	11 ± 0.2	11 ± 0.0	12 ± 0.5
Tribulus terrestris	L	10 ± 0.0	12 ± 1.0	19 ± 1.5	21 ± 0.6	21 ± 1.2	22 ± 0.6
Trigonella foenum-graecum	Se	10 ± 0.3	10 ± 1.0	10 ± 0.2	11 ± 0.3	10 ± 0.0	13 ± 0.6
Zizyphus spina-christi	L	14 ± 1.2	17 ± 2.6	10 ± 0.0	10 ± 0.0	10 ± 0.0	10 ± 0.6
Zizyphus spina-christi	B	15 ± 1.0	19 ± 0.5	25 ± 1.5	34 ± 0.6	25 ± 2.5	27 ± 3.0

B = Barks; L = Leaves; Se = Seeds; F = Fruits; R = Root; S = Stems and WA = Whole Aerial parts.
E.c. = *Escherichia coli*, S.a. = *Staphyllococcus aureus*, K.p. = *Klebsiella pneumoniae*. I.Z.D. = Mean diameter of growth inhibition zones in mm average of three replicates, SD = Standard deviation.

against NDV. Seven of these active plants extract had shown complete inhibition as recorded in Table (3). Moreover, eleven plant extracts exhibited high activity against PV (Table 3).

The activities demonstrated by the different types of extracts may be attributed to the diversity of structures and/or the uneven distribution of chemical constituents within these extracts. Each extract had a different degree of inhibitory activity and specificity against the virus and/or its essential enzymes. Also, it may be due to the fact that, most of the plants showing antiviral activity were found to contain some proteinaceous substances or polypeptide as reported by Bajpai and Chandra (1990). These plant proteins have been reported to act against the viruses by inhibiting their protein synthesis.

Antiviral phytochemicals were profoundly affected by various reaction parameters as was reported by Hudson et al. (1994) who found that, the activities of several known antiviral phytochemicals were affected by the presence of serum components. These findings are significant because serum is commonly used in virus assays, and plant extracts often contain polypeptides. Furthermore, when phytochemicals are used in vivo, their effects could be modulated by the components of tissues and body fluids. The reactions are also strongly affected by the order of incubation of the components, virus compounds or extracts, serum, and UV-light. The bioassay technique followed may also have a great affect on the inhibitory potential. Antivirals as well as other compounds with indirect antiviral activities may have various mechanisms of action.

Table 3. The antiviral activity of MeOH extracts of selected plants from Sudan.

Botanic name	Part screened	HA inhibition %		Inhibition of pock lesions formation	
		100 μg/mL	200 μg/mL	100 μg/mL	200 μg/mL
Abutilon figarianum	L	7	0	+ +	+ +
Acacia nilotica	F	0	7	+ + +	+ + +
Aloe sinkitana	L	22.5	22.5	+ +	+ +
Aloe sinkitana	R	0	0	+	+
Aristolochia bracteolate	L	7	57.5	+ +	+ +
Aristolochia bracteolate	F	15	22.5	+ + +	+ + +
Avicennia marina	L	15	22.5	+	+
Avicennia marina	S	100	100	+ + +	+ + +
Cassia obtusifolia	L	0	0	i	i
Caralluma retrospiciens	WA	22.5	22.5	+ + +	+ + +
Cissus quadrangularis	WA	22.5	7	+ + +	+ + +
Combretum glutinosum	B	100	100	i	i
Coriandrum sativum	F	0	0	i	i
Croton zambesicus	F	7	0	+ +	+ +
Diospyros mespiliformis	B	100	100	i	i
Diospyros mespiliformis	L	100	100	+ +	+ +
Harrisonia abyssinica	L	30	7	+ + +	+ + +
Harrisonia abyssinica	F	15	7	+ + +	+ + +
Ipomoea carnea Jacq.	L	7	0	+ +	+ +
Lavandula coronopifolia	WA	100	100	+ +	+ +
Maerua oblongifolia	L	15	7	+ + +	+ + +
Maytenus senegalensis	L	0	0	+	+
Maytenus senegalensis	B	22.5	0	+	+
Nigella sativa	Se	100	100	+	+
Prosopis chilensis	L	30	15	+ + +	+ + +
Tamarindus indica	F	0	0	i	i
Tribulus terrestris	L	0	0	+ + +	+ + +
Trigonella foenum-graecum	Se	0	7	+ +	+ +
Zizyphus spina-christi	L	22.5	0	+	+
Zizyphus spina-christi	B	100	100	+	+

L = Leaves, S = Stem, B = Barks, WA = Whole Aerial parts, F = Furits, Se = Seeds, R = Roots
+ + + = highly active, + + = moderate, + = mild, i = inactive

Table 4. The phytochemical screening of MeOH extracts of selected plants from Sudan.

Botanic name	Alkaloids	Saponins	Triterpenes/ steroids	Flavonoids	Tannins
Abutilon figarianum	-	-	+	+ + +	+
Acacia nilotica	+	-	+ +	+	+ + +
Aloe sinkitana	-	+	-	+	-
Aristolochia bracteolate	-	+	-	+	+
Avicennia marina	-	-	+	+	-
Cassia obtusifolia	+	+	-	+	+
Caralluma retrospiciens	-	+	+	+ +	-
Cissus quadrangularis	-	+	+	+ +	-
Combretum glutinosum	-	+ + +	+ +	+ +	+
Coriandrum sativum	-	+	+ +	+	-
Croton zambesicus	-	+	+ +	+ + +	-
Diospyros mespiliformis	-	+ +	+ + +	+	+
Harrisonia abyssinica	+	+	+	+ +	-
Ipomoea carnea	+ + +	+ +	+	+	-
Lavandula coronopifolia	-	-	+ +	+	-
Maerua oblongifolia	-	+ +	+ +	+ +	-
Maytenus senegalensis	+	+	+ + +	+	+
Nigella sativa	-	-	+	+ + +	-
Prosopis chilensis	-	+	+	+ +	+
Tamarindus indica	-	+	+ +	+ +	+
Tribulus terrestris	-	+ +	-	+ + +	+
Trigonella foenum-graecum	-	+	-	+ +	-
Zizyphus spina-christi	+	+ + +	+	+ + +	+
Reference compound	+ + +	+ + +	+ + +	+ + +	+ + +

+ + +, high concentration; + +, medium concentration; +, low concentration; -, not detected
Reference compounds: Quinine, furostanol, lupeol/ß-sitosterol, vitexin, gallic acid, respectively.

Viral infections are usually accompanied by a variety of symptoms not necessarily due to the virus directly, and it is possible that there are other ingredients in a plant preparation that help to control the virus by additional effects, such as immune modulation, tissue-healing, etc. Virus infections are frequently accompanied by disturbances in immune functions and other important metabolic pathways, thereby, influencing multiple physiological parameters. Furthermore, the maximum beneficial effect of a medicinal plant preparation may require the synergistic contribution of antiviral in addition to the other activities. Traditional healers usually give a mixture of some plants for the treatment of diseases; the mixture could be active due to synergistic effects (Gessler et al. 1994).

The phytochemical results of all tested plant species are given in Table (4).

CONCLUSION

The various traditional uses of the majority of tested plants correlate well with our findings. The results of this preliminary evaluation give evidence that some of the ethnobotanically selected and traditionally used Sudanese plant species can be regarded as promising resources for antibacterial and/or antiviral drugs. It seems that further investigations are necessary in order to draw solid conclusions.

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Bazi Sudan Tibbi Bitkilerinin Antibakteriyel ve Antiviral Etkileri ve Fitokimyasal Taranmasi

Özet

19 familyaya ait 23 farkli bitkinin 30 parçasindan metanolik özütler elde edildi. Bitkilerin çogu Sudan'da çesitli hastaliklari tedavi etmek için kullanilmaktadir. Özütlerin; *Escherichia coli, Staphyllococcus aureus,* ve *Klebsiella pneumoniae* gibi bakteriyel patojenlere ve iki virüs familyasini temsilen iki hayvan virüsüne, Newcastle hastaligi ve tavuk çiçegine karsi biyolojik altiviteleri ölçüldü. Buna ek olarak, özütler ana sekonder metabolit siniflarinin mevcudiyeti açisindan test edildi. En yüksek aktivite, *S. aureus* ve *K. pneumoniae*'e karsi *Zizyphus spina-christi*'de görüldü. Test edilen özütlerin yedisi NDV'ye karsi virüsidal aktivite sergilerken, sekiz özüt PV replikasyonuna karsi yüksek aktivite gösterdi. Sonuçlarimiz çogu bitkinin etnofarmakolojik kullanim iddialarini, kismen de olsa, desteklemektedir.

Anahtar Kelimeler: Antibakteriyel, antiviral, bitki, Newcastle hastalik virusü, Sudan, tavuk çiçegi.

